SANDAKANIA 12 (1998): 27–36

Initial colonisation and prey capture in *Nepenthes bicalcarata* (*Nepenthaceae*) pitchers in Brunei

Charles Clarke

Department of Biology,
Hong Kong University of Science and Technology,
Clear Water Bay Road,
Kowloon, Hong Kong

Summary. Initial prey capture and colonisation of pitchers of the carnivorous plant, *Nepenthes bicalcarata* were examined in Brunei. It was found that the pitchers possess large nectaries on their external surfaces which are very active during pitcher development and are attended by numerous species of ants, which also comprise a significant proportion of the pitchers' prey once they open. The inquiline ants (*Camponotus schmitzi*) of *N. bicalcarata* do not interact with the ants which attend the external nectaries, only colonising the pitchers as they open. At this time, the other ants cease to attend the external nectaries, feeding at those around the pitcher rim and lid instead. *C. schmitzi* ants exhibit a preference for aerial pitchers, whereas the ants which attend the nectaries do not. Prey capture and colonisation of the pitchers by inquiline metazoan species occurs as soon as the pitchers open, but the majority of colonisation/capture events take place during the second week after opening. Pitcher type (aerial or terrestrial) has no effect on prey capture or colonisation by infaunal species. The presence of *C. schmitzi* affects numbers of infaunal species, perhaps because these ants prey upon members of the pitcher fauna. Prey numbers and mass are not affected by *C. schmitzi*.

Nepenthes bicalcarata (Hook. f.) is a carnivorous pitcher plant common in the peat swamp forests of north-western Borneo. Like most other *Nepenthes*, this species is a climbing vine which produces two types of pitcher, depending on the age of the plant. Young rosettes produce large, squat, urceolate pitchers that hold large quantities of fluid. These usually rest on the ground and are referred to as terrestrial or lower pitchers. Climbing stems produce smaller pitchers which are more infundibular and contain correspondingly small volumes of fluid. These are the aerial or upper pitchers. Ants comprise the bulk of prey in most *Nepenthes* and *N. bicalcarata* is no exception to this pattern (see Jebb 1991, Moran 1996, Clarke 1997a). The fluid of *N. bicalcarata* pitchers is usually acidic (pH ~ 4.5) and a large number of invertebrate species have been recorded living in the pitchers (Clarke & Kitching 1993). *N.*

bicalcarata is unique among pitcher plants in that its pitcher tendrils are hollow and are colonised by *Camponotus schmitzi* (Schuitemaker & Stärke) ants. These ants forage by swimming in the pitcher fluid and retrieving captured insects which they consume. A detailed account of this ant–plant association is provided by Clarke & Kitching (1995).

Clarke (1997a) provided a brief description of pitcher development in *N. bicalcarata*, showing that the pitchers take approximately 27 days to develop from a small bud to an open, functioning pitcher. *N. bicalcarata* pitchers have an average operational lifespan of about 230 days, but may live for over a year (Clarke 1997a). The pitchers have several large nectaries on the external surfaces. These are very active as the pitcher develops and are attended by numerous species of ants, but not by *C. schmitzi* (Clarke & Kitching 1995).

Despite recent detailed accounts of the prey and food webs in *Nepenthes* pitchers (see Jebb 1991, Kato *et al.* 1993, Moran 1996, Beaver 1979, 1983, 1985, Clarke & Kitching 1993, Mogi & Chan 1996, 1997), little is known about patterns of prey capture and pitcher colonisation during pitcher development and the period immediately after the pitchers open. This paper presents the results of experiments and observations designed to investigate the initial colonisation and prey capture patterns in *N. bicalcarata* pitchers. The effects of various factors, including pitcher type (upper or lower), the presence or absence of *C. schmitzi* ants and time upon variables such as numbers and species of infaunal organisms, and numbers and mass of prey are examined. The objective is to provide both a qualitative and a quantitative overview of these processes, forming a basis upon which further experiments can be performed.

STUDY SITE

The study site was along a 3-km stretch of an abandoned logging railway behind the township of Seria in the Belait District of Brunei Darussalam. The vegetation in this area is "alan bunga" peat swamp forest, which is dominated by the dipterocarp, *Shorea albida* Sym. This forest type is described in Whitmore (1984).

MATERIALS AND METHODS

New leaves with undeveloped pitcher buds from 41 *N. bicalcarata* plants were labeled at the site. The buds were inspected for seven weeks between October 19 and December 5, 1989. Each bud was numbered and where referred to in this text, they are denoted as such: # 2, # 16, # 37, etc.

Buds were observed to see whether nectar feeding ants were present or absent at the external nectaries. Pitcher type (upper or lower) was recorded, as was the presence or absence of the inquiline ants (*C. schmitzi*). If *C. schmitzi* colonies were established in the pitcher tendrils, the time between the start of pitcher development and colonisation was measured to the nearest day. Any evidence of herbivory on the developing pitchers was recorded.

The pitchers were observed for three weeks after opening, during which three sets of pitchers were removed and their contents analysed. Ten pitchers were examined one week after opening, nine pitchers were examined after two weeks and ten pitchers were examined after three weeks. Each of the 29 pitchers was removed intact from the plant and taken to the laboratory for examination. The number of prey items was counted, along with the number of infaunal species which had colonised each pitcher.

The dry weight of the prey was measured by removing all of the macroscopic infaunal organisms and draining the fluid from the pitcher contents through a piece of filter paper. Each piece of filter paper used was labeled and placed in a drying oven for two weeks prior to the experiment. When required, each paper was removed from the drying oven and immediately weighed to the nearest 0.0001g. After draining the pitcher contents, each paper (and contents) were returned to the drying oven for a period of ten days, after which time they were weighed again.

Descriptive statistics for each pitcher characteristic were calculated, with all means stated in the text being means ± 1 standard error (SE). Distributions of ordinal measures (e.g., presence/absence of ants, upper/lower pitchers) were compared using χ^2 homogeneity tests. One-way analysis of variance was used to test for significant differences among ratio measures (e.g., dry weight of prey, number of days to opening) for each class of pitcher (upper/lower, colonised/uncolonised by *C. schmitzi*).

One- and two-way analyses of variance were performed, using the following factors: pitcher type (upper/lower), presence/absence of *C. schmitzi* and week number (1, 2 or 3) against the following variables: numbers of infaunal species, numbers of infaunal organisms, numbers of prey organisms, and prey mass. Only results which were significant at $\alpha = 0.05$ are presented here.

OBSERVATIONS AND RESULTS

Of the 41 buds examined, two failed to develop (# 2 and # 6) and one (# 38) was either developing unusually slowly, or was failing to develop fully when the experiment was concluded (Fig. 1). Bud # 2 was damaged by herbivores during the second week of observations. Herbivory seems to be very rare in *N. bicalcarata* and this was the only evidence of it detected during the study. The external nectaries on the developing pitcher buds were attended by a variety of ants. These species were also captured by the pitchers after opening. Thirty of the buds were observed to be attended by ants during the experiment. Whether the remaining 11 buds were attended at other times, or not at all, is unknown. Of the 38 pitchers which developed fully, there were 19 upper and 19 lower pitchers. The χ^2 homogeneity test to compare the frequencies of upper and lower pitchers which were attended by ants showed that these frequency distributions are not independent ($\chi^2 = 0.0084$, 1 d.f., $p > 0.05$, $\alpha = 0.01$). Fig. 1 shows the patterns of observed attendance throughout the observation period, coupled with the time of colonisation of the pitchers by *C. schmitzi*.

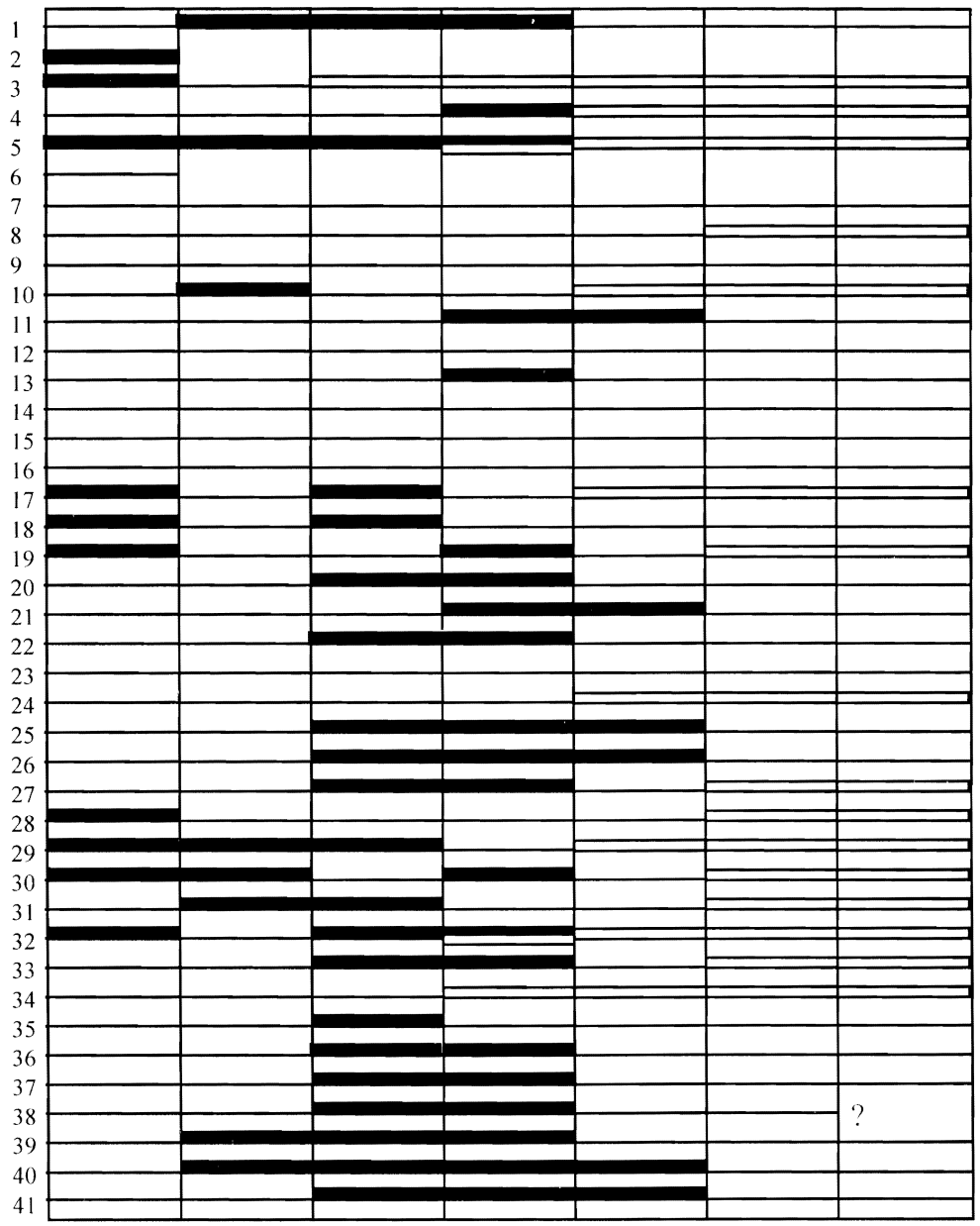

Fig. 1. Attendance of developing pitcher buds throughout the observation period.
Numbers along the left denote individual pitcher buds observed. Thin lines denote continuing development of pitcher buds. Cessation of the thin line indicates that the pitcher did not complete development. Thick, solid lines indicate observation weeks in which ants other than *C. schmitzi* were observed feeding from the nectaries on the external surfaces of the pitchers. Thick, shaded lines indicate the sampling periods when the pitchers were observed as being colonised by *C. schmitzi*. It was not possible to determine whether pitcher #38 had ceased development, or whether it was growing at an unusually slow rare.

Two patterns are apparent from Fig. 1. First, the external nectaries of the buds were regularly seen to be attended by ants other than *C. schmitzi* for the first four weeks of development. However, only six buds were attended during the fifth week and none were attended after that date. It is possible that the sampling method failed to detect the attendance of some buds by ants—no nocturnal observations were made and each pitcher could only be observed for a limited time on each visit to the field site. Nevertheless, that no ants were observed at these nectaries on any of the 41 buds after the fifth week suggests that the external nectaries become less attractive to foraging ants as the pitchers open. Whether this is because these nectaries cease or reduce their nectar output, or whether the ants are more attracted by the new sources of nectar produced by the glands inside the pitchers (which become operational as the pitchers open) is not known.

Second, there is a clear demarcation of attendance by nectar feeding ants and the arrival of *C. schmitzi* at the pitchers. This also coincides with the time at which the pitchers opened. Only two of the pitchers (# 5 and # 32) were in the process of being colonised by *C. schmitzi* at the same time other ants were seen at the external nectaries (during week 4). *C. schmitzi* ants did not exhibit any aggressive behavior towards any organisms outside the pitcher fluid so it seems unlikely that the ants attending the nectaries on the outer surfaces of the pitcher were driven away by them.

C. schmitzi colonised 16 of the 38 buds which developed into functioning pitchers during the observation period, showing a significant preference for upper pitchers over lower ones (χ^2 = 14.401, 1 d.f., $p < 0.001$, $\alpha = 0.01$). Only one pitcher (# 3) was attended by *C. schmitzi* before the fourth week of observations, with most pitchers being colonised during the sixth week. Clarke & Kitching (1995) suggested that the optimal time for colonisation by *C. schmitzi* is during the period immediately after the pitchers open: any earlier and the size of the hole bitten by the ants would become too large as the pitcher develops, perhaps leaving them vulnerable to predators. Any later and the tissues of the tendril would become so hard that the ants might have difficulty biting through them. These results support that interpretation.

Prey types and numbers, infaunal species and numbers, and pitcher types are presented in Table 1. Pitcher type (upper or lower) had no significant effects upon any of the variables examined. The only significant effect of the presence/absence of *C. schmitzi* was upon numbers of infaunal species (F = 6.935, d.f. = 1, 27, $p = 0.014$, $\alpha = 0.05$). Time (weeks 1–3) had significant effects upon numbers of infaunal organisms (F = 3.568, d.f. = 2, 26, $p = 0.043$, $\alpha = 0.05$) and prey mass (F = 5.168, d.f. = 2, 26, $p = 0.013$, $\alpha = 0.05$). There were no significant interactions between factors. All other combinations of factors and variables returned non-significant results at a = 0.05.

DISCUSSION

Attendance of external nectaries by ants

Although it is not yet possible to determine the exact role of the external nectaries on *N. bicalcarata* pitchers, it is likely that they serve to attract ants other than *C. schmitzi* for one of

Table 1. Descriptive statistics for pitcher variables.
All values are means ± 1 SE and the values for each variable were determined on a "per pitcher" basis.

Factor		Variable			
		No. of prey items	**Prey mass**	**No. of infaunal species**	**No. of infaunal organisms**
Time					
	week 1	7.500 ± 1.614	0.0320 ± 0.0030	3.200 ± 0.291	24.100 ± 4.608
	week 2	28.667 ± 3.958	0.0500 ± 0.0050	3.556 ± 0.338	74.333 ± 20.438
	week 3	42.300 ± 17.650	0.0490 ± 0.0060	4.200 ± 0.442	55.100 ± 11.930
C. schmitzi					
	present	14.000 ± 3.086	0.0429 ± 0.0042	3.077 ± 0.178	44.154 ± 10.114
	absent	35.875 ± 11.296	0.0439 ± 0.0043	4.125 ± 0.328	55.438 ± 12.945
Pitcher type					
	upper	15.667 ± 3.843	0.0433 ± 0.0038	3.467 ± 0.307	41.133 ± 9.099
	lower	37.214 ± 12.640	0.0436 ± 0.0047	3.857 ± 0.312	60.286 ± 14.267

the two reasons outlined above (i.e., to establish the pitcher as a source of nectar and/or to protect the pitchers from attack by herbivores while they develop) (see Clarke 1997a). Either theory remains plausible at this stage, and it may be that the external nectaries serve a dual purpose. The cessation of attendance of the external nectaries can be explained by both hypotheses: once the pitchers are open, nectar is most abundant within the pitcher and on the lid. Hence foraging ants will be attracted to those areas rather than the external nectaries. Similarly, if the role of the ants is to deter other animals from visiting the developing pitcher, this role would cease once the pitchers open if the plant is to catch sufficient prey. However, nectar production rates by the external nectaries have not been determined, and further experiments would provide better insights.

Selective colonisation of upper pitchers by C. schmitzi

Clarke & Kitching (1995) suggested that *C. schmitzi* colonise upper pitchers in preference to lower ones because the nest chambers of the lower pitchers may be subject to flooding after heavy rain. Although no quantitative data were provided to support this claim, these observations show that *C. schmitzi* does have a distinct preference for upper pitchers, and those lower pitchers which are held high above the ground by supporting vegetation are usually colonised by *C. schmitzi* (C. Clarke, pers. observ.), suggesting that avoidance of flooding is the reason why upper pitchers are colonised more than lower ones.

The effects of pitcher colonisation by C. schmitzi

Clarke & Kitching (1995) showed that the behaviour of *C. schmitzi* only has a detectable effect on pitcher contents when excess prey is caught. These analyses support this finding. Although *C. schmitzi* remove large prey to feed, the remains are returned to the pitcher, so their activities can not be quantified in this way: direct observation of their feeding behaviour is required. Numbers of infaunal organisms are also unaffected, but there are significantly fewer infaunal species present in pitchers colonised by *C. schmitzi*. Mogi & Chan (1997) suggested that the presence of aquatic predators in pitcher communities serves to increase overall species numbers, with the predators' feeding behaviour reducing the numbers of the most common prey species, thereby allowing less competitive species to colonise the pitchers. Although this survey lasted only three weeks, these results do not support that interpretation. It is possible that *C. schmitzi* preferentially feed on the aquatic predators, thus reducing selective pressure on their prey species, but this can not be substantiated at present. It is difficult to determine whether the results obtained in this experiment reflect the overall situation for *N. bicalcarata* pitchers without the aid of further studies.

The effects of pitcher dimorphism

Clarke (1997b) showed that pitcher dimorphism in *N. bicalcarata* has no effect on food web structure. In contrast, Moran (1996) found that pitcher dimorphism in *N. rafflesiana* (Jack) does affect the prey spectrum of that species. In *N. bicalcarata*, it appears that pitcher dimorphism is not an important determinant of either prey capture or colonisation by

metazoan species, as even the smallest fluid volumes in the upper pitchers are relatively large for *Nepenthes* (Clarke & Kitching 1993) and the pitchers are long-lived (Clarke 1997a). Although the results presented here support the findings of Clarke (1997b), it is important to emphasise that inter-specific differences in *Nepenthes* could be important in this regard and that the patterns evident in *N. bicalcarata* may not apply to other species.

The effects of time

Over the three weeks in which the open pitchers were examined, prey mass and numbers of infaunal organisms varied significantly with time. As pitchers accumulate prey and detritus after opening, it is not surprising that prey mass increases as the pitchers age. The same situation exists for numbers of infaunal organisms: colonisation of newly opened pitchers takes place over several weeks (Clarke 1992). However, for both of these variables, there is a sharp increase in mass and numbers from weeks 1–2, but very little further increase from weeks 2–3. This suggests that the pitchers catch little and are colonised by few species during the first few days after opening, but there is a burst of trapping and colonisation during the second week. Why this burst is not sustained into the third week is difficult to determine without subsequent measurements.

The prey spectrum of *N. bicalcarata* pitchers during the first three weeks is quite narrow, with all organisms falling into one of five orders (in decreasing order of abundance): Hymenoptera, Coleoptera, Hemiptera, Aranae and Orthoptera. In contrast, Moran (1996) found 11 orders of prey in *N. rafflesiana* pitchers in Brunei, but those experiments included much larger numbers of pitchers, of varying ages. Whether the corresponding burst in colonisation by infaunal species is related to increased prey capture is also unknown. Given that infaunal species numbers vary little throughout this period, it seems likely that a small subset of the *N. bicalcarata* community colonises pitchers repeatedly during the first few weeks of the pitchers' lives, with the other species arriving later.

CONCLUSIONS

The development of *N. bicalcarata* pitchers is a complex process which involves an array of invertebrates, many of which fulfill different roles for the plant. Many of these roles are not presently known or fully understood, such as that of the ants which attend the nectaries of the developing pitchers (but which are then caught by the pitchers after they open). The stark demarcation of pitcher attendance and colonisation by *C. schmitzi* is clearly linked to the opening of the pitchers, but the reasons for the demarcation are far from clear. Although *C. schmitzi* have a distinct preference for colonising aerial pitchers, there are no discernible patterns in the attendance of the nectaries by the other ants, so perhaps their behaviour is determined more by the opening of the pitchers rather than any interaction among these ants and *C. schmitzi*.

The capture of prey by the pitchers shows an increase in mass and numbers over the initial three weeks, but the observation period was clearly too short to determine whether there are

any patterns in prey capture. The infaunal species which colonised the pitchers belonged to a small group of species which colonise the pitchers repeatedly soon after they open. As with prey capture, further observations are required to determine whether this is a part of a distinct pattern or simply an artifact of the sampling procedure.

ACKNOWLEDGEMENTS

I thank the Brunei Research Council for permitting me to carry out research on *Nepenthes* in Brunei Darussalam. I thank Dr Webber Booth and Professor Roger Kitching for their assistance and advice in relation to my research, which was supported by an Australian Postgraduate Research Award, administered by the University of New England, Armidale, Australia.

REFERENCES

Beaver, R.A. (1979) Fauna and foodwebs of pitcher plants in West Malaysia. *Malayan Nature Journal* 33: 1–10.

———— (1983) The communities living in *Nepenthes* pitcher plants: fauna and foodwebs. In: J.H. Frank & L.P. Lounibos (eds.), Phytotelmata: terrestrial plants as hosts for aquatic insect communities, pp. 129–159. Plexus Publishing, Marlton, New Jersey.

———— (1985) Geographical variation in food web structure in *Nepenthes* pitcher plants. *Ecological Entomology* 10: 241–248.

Clarke, C.M. (1992) *The ecology of metazoan communities in Nepenthes pitcher plants in Borneo, with special reference to the community of* Nepenthes bicalcarata *Hook. f.* Ph.D. Thesis, University of New England, Armidale, New South Wales, Australia.

———— (1997a) *Nepenthes of Borneo*. Natural History Publications (Borneo), Kota Kinabalu.

———— (1997b) The effects of pitcher dimorphism on the metazoan community of the carnivorous plant *Nepenthes bicalcarata* Hook. f. *Malayan Nature Journal* 50: 149–157.

Clarke, C.M. & R.L. Kitching (1993) The metazoan food webs from six Bornean *Nepenthes* species. *Ecological Entomology* 18: 7–16.

———— (1995) Swimming ants and pitchers plants: a unique ant–plant interaction from Borneo. *Journal of Tropical Ecology* 11: 589–602.

Jebb, M.H.P. (1991) An account of *Nepenthes* in New Guinea. *Science in New Guinea* 17 (1): 7–55.

Kato, M., M. Hotta, R. Tamin & T. Itino (1993) Inter- and intra-specific variation in prey assemblages and inhabitant communities in *Nepenthes* pitchers in Sumatra. *Tropical Zoology* 6: 11–25.

Mogi, M. & K.L. Chan (1996) Predatory habits of dipteran larvae inhabiting *Nepenthes* pitchers. *Raffles Bulletin of Zoology* 44(1): 233–245.

———— (1997) Variation in communities of dipterans in *Nepenthes* pitchers in Singapore: predators increase prey community diversity. *Annals of the Entomological Society of America* 90 (2): 177–183.

Moran, J.A. (1996) Pitcher dimorphism, prey consumption and the mechanisms of prey capture in the pitcher plant *Nepenthes rafflesiana* in Borneo. *Journal of Ecology* 84 (3): 515–525.

Whitmore, T.C. (1984) *Tropical Rain Forests of the Far East.* 2nd edition. Oxford University Press, Oxford, England.